FIXATION PROCEDURE FOR CHICKEN BLASTODERMS

Washington State University
Zoology Dept.
James D. Huber

The shell is cracked and the egg is gentley allowed to slip into a square petri dish. Normally the blastoderm will appear at the top, but there may be times when it will be on the side or bottom. This can be corrected by sliding a piece of parafilm under the yoke and carefully lifting. The yoke will rotate and the blastoderm can be positioned to the top. The albumin is removed from over the vitelline membrane, by slipping one tip of a round nose sissors under the albumin and against the vitelline membrane. When the albumin is cut, it will slip off the vitelline membrane, and expose the blastoderm. A 4mm ring is cut from the top of a standard plastic BEEM embedding capsule and set on top of the vitelline membrane (surrounding the blastoderm) and the fixative solutions put into the well. The glutaraldehyde fixative(0.5% in 0.1M phosphate buffer pH-7.4) is allowed to remain for 5-15 min.and then pipeted out of the well and immediately replaced by the osmium tetroxide solution (60-80% in carbon tetrachloride). A cap from the cut BEEM capsule is inverted and placed over the top of the ring to keep the osmium vapor inside the well. After the fixation is completed, the first step of dehydration (30% alcohol) is also begun in the well. The plastic ring will now adhere to the vitelline membrane and the is cut around the outside of the ring with sissors and the ring is lifted free from the yoke. All the excess yoke is drained from the under side of the blastoderm and any remaining amount is wiped with a piece of lens tissue. The remaining dehydration and embedding steps are now continued in small vials. The plastic ring is left attached to the vitelline membrane until after the epon infilltration to assure that the vitelline membrane will not fold during the dehydration and distort the blastoderm cells.

We have found that the non-incubated, incubated and stored blastoderm cells can be stabilized well with 0.5% glutaraldehyde for 5-30 min. at room temperature with a phosphate buffer of 0.1M and a total fixation osmolarity of about 250. Postfixation is with 40-80% osmium tetroxide in carbon tetracholride for 2-5 min. Dehydration is begun immediately after removal of the osmium in a alcohol series of 30,50,70,95% for 10 min. each and 100% 2 changes of 5 min. each. Propylene oxide replaces the alcohol with two changes of 10 min. each and a epon/propylene oxide mixture (1:1) is infiltrated into the tissues. The propylene oxide is allowed to evaporate (cap off) in a hood over night and then replaced with fresh epon and embedded. The plastic ring is removed from the blastoderm at the time of embedding.
We have also found that 5% osmium tetroxide in 0.1M phosphate buffer can be used as a postfixative, but the membrane definition(probably osmium staining) is not as good as with the stronger osmium. Also we have found that blastoderms fixed in the cold or dehydrated with cold alcohols have shown a loss of microtubule formation.

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